Received: 28 November 2022 | Accepted: 31 December 2022

DOI: 10.1002/cia2.12294

Journal of Cutaneous Immunology and Allergy

WILEY

CASE STUDY

Severity and intractableness of skin infections caused by Panton–Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus*

Tamihiro Kawakami MD, PhD[1] | Kae Yokoyama MD[1] | Takaharu Ikeda MD, PhD[1] | Hiroshi Kaneko MD, PhD[2] | Yuji Watanabe PhD[3] | Hidemasa Nakaminami MD, PhD[2]

[1]Division of Dermatology, Tohoku Medical and Pharmaceutical University, Sendai, Japan

[2]Department of Clinical Microbiology, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan

[3]Division of Clinical Laboratory, Tohoku Medical and Pharmaceutical University Hospital, Sendai, Japan

Correspondence
Tamihiro Kawakami, MD, PhD, Division of Dermatology, Tohoku Medical and Pharmaceutical University, 1-15-1 Fukumuro, Miyagino-ku, Sendai, Miyagi 983-8536, Japan.
Email: tami@tohoku-mpu.ac.jp

Abstract

Cases of skin infections caused by Panton–Valentine leukocidin (PVL)-positive methicillin-resistant *Staphylococcus aureus* (MRSA), particularly USA300 clone, have been increasing in Japan. We report that clinical findings of 5 patients with PVL-positive MRSA and compared to those of four patients with PVL-negative MRSA. Severities of patients with PVL-positive MRSA were significantly higher than those of patients with PVL-negative MRSA. Average durations of antimicrobial therapy for patients with PVL-positive MRSA were 3.4-fold longer than those for patients with PVL-negative MRSA. Our data suggest that PVL-positive MRSA should be deal with a causative agent for intractable skin infections in Japan likewise other countries.

KEYWORDS
methicillin-resistant *Staphylococcus aureus*, Panton–Valentine leukocidin, USA300

1 | INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are classified clinically into two types: hospital-acquired MRSA and community-acquired MRSA (CA-MRSA). The spread of CA-MRSA has become a serious problem worldwide. The global distribution of CA-MRSA clones is heterogeneous and often characterized by a regional predominant clonal lineage.[1] Recently, there have been increasing reports of Panton–Valentine leukocidin (PVL)-positive MRSA from not only medical facilities but also from the community in Japan.[2] PVL is a bicomponent pore-forming toxin associated with clinical diseases, particularly deep-seated skin infections and necrotizing pneumonia.[1]

USA300 clone, which produces PVL, is one of the highest pathogenic and global epidemic CA-MRSA strains that causes intractable skin infections. This epidemic clone has the following genetic features: staphylococcal cassette chromosome *mec* (SCC*mec*) type IV, PVL-positive, arginine catabolic mobile element (ACME) type I, and clonal complex (CC) 8.[3] Reports of skin infections caused by the USA300 clone have recently been increasing in Japan, and the USA300 clone has disseminated in both community and hospital settings.[4–6] Generally, PVL-positive MRSA is considered as causative pathogen of severe skin infections.[1] However, little is known whether PVL-positive MRSA is associated with intractable skin infections rather than PVL-negative MRSA in Japan. Here, we report

that clinical findings of patients with PVL-positive MRSA and compared to those of patients with PVL-negative MRSA.

2 | PATIENTS AND METHODS

2.1 | Patient 1

A 90-year-old Japanese man who had liver cirrhosis and chronic lymphocytic leukemia presented with severely inflamed abscesses in his right arm (Figure 1A). It was diagnosed as carbuncle and treated with 1 week of vancomycin intravenous drip infusion. MRSA was identified in cultures of exudate from the carbuncle.

2.2 | Patient 2

A 47-year-old Japanese man who had diabetes, diabetic nephropathy, and myocardial infarction presented with sudden onset of redness with abscesses in his right foot. He had a high fever. Physical examination revealed painful erythematous lesions in the swelling of his right leg (Figure 1B). MRSA was identified in cultures of exudate from the abscesses. Although we suspected carbuncle, he was not administered antibiotics because he rejected hospitalization. One week later, he developed blackish discoloration of the toes and swelling of the right leg. The laboratory data were as follows: leukocytes 30,000/μl and CRP 28.76 mg/dl. He was diagnosed with necrotizing fasciitis and treated with 30 days of teicoplanin intravenous drip infusion. Gangrene was also found, and he subsequently underwent amputation.

2.3 | Patient 3

A 61-year-old Japanese man presented with inflamed abscesses in his lower extremities. Physical examination revealed painful carbuncles in the swelling of the legs (Figure 1C). It was diagnosed as carbuncle and treated with 1 week of vancomycin intravenous drip infusion. MRSA was identified in cultures of exudate from the carbuncle in his right leg.

2.4 | Patient 4

A 59-year-old Japanese man with atopic dermatitis presented with a complaint of repeated cutaneous infections on his legs and trunk (Figure 1D). The patient was diagnosed with furuncle and treated by incision of the furuncles and 2 weeks of oral minocycline. MRSA was identified in cultures of exudate from the furuncle of the buttock.

2.5 | Patient 5

A 23-year-old Vietnamese man presented with a purple-red raised lesion with abscesses on his limbs. Physical examination revealed a 4-cm diameter nodule in the center and an orifice with purulent discharge on his left lower limb. The patient was diagnosed with furuncle and treated with 2 weeks of oral fosfomycin with gentamicin sulfate ointment. MRSA was identified in cultures from the furuncle on his left leg.

2.6 | Molecular epidemiological analysis

The following *S. aureus* strains were used as SCC*mec* type strains: NCTC10442 (type I), N315 (type II), 85/2082 (type III), JCSC4744 (type IV), and WIS (type V).[7] MRSA strains JCSC6774 and N315 were used as a standard strain of the USA300 clone and a reference strain for pulsed-field electrophoresis (PFGE), respectively.[8] PCR assays for the detection of *mecA*, PVL genes (*lukS/F-PV*), toxic shock syndrome toxin-1 (TSST-1) gene (*tst*), and ACME genes (*arcA* and *opp-3C*) were carried out as described previously.[4] SCC*mec* typing, multilocus sequence typing (MLST), and PFGE analyses were also performed as described previously.[4]

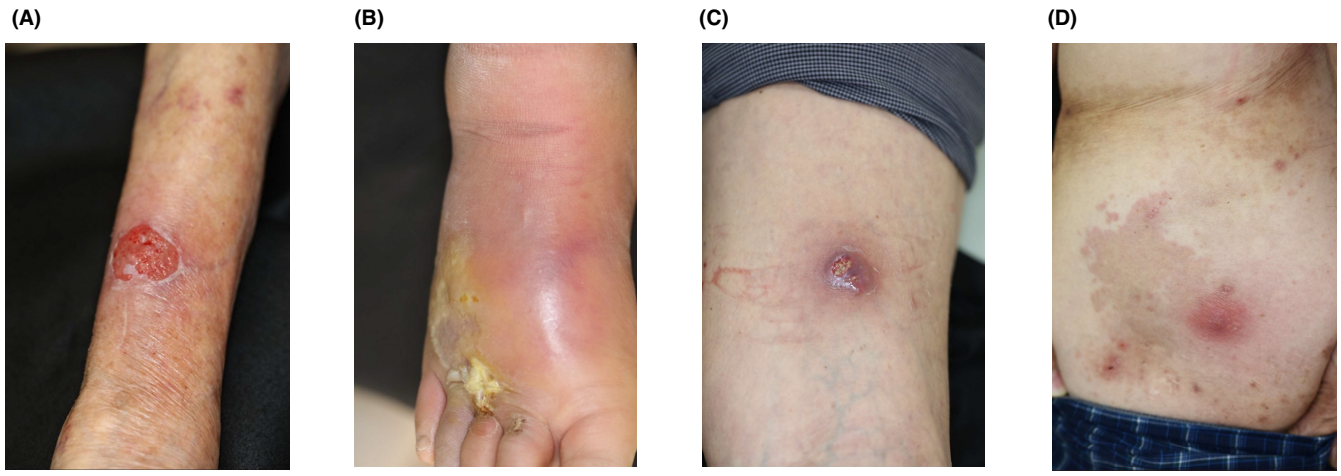


FIGURE 1 Physical examination in PVL-positive MRSA patients. (A) Patient 1 right arm, (B) patient 2 right foot, (C) patient 3 right leg, and (D) patient 4 buttock

The study protocol was approved by the ethics committee of Tohoku Medical and Pharmaceutical University (2022–2-040). Informed consent from the original patients was not required because the study did not involve clinical interactions with the patients.

3 | RESULTS

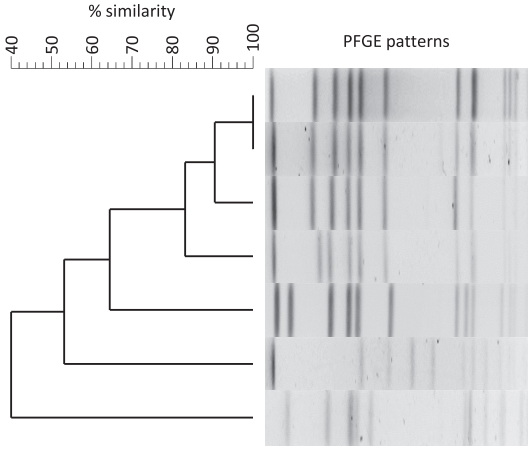
We recruited patients with MRSA ($n = 9$) from those who visited our Dermatology Department between June 2020 and January 2022. Molecular epidemiological analyses revealed that PVL-positive MRSA strains were identified in the cutaneous lesions of patients 1–5. The patients comprised five males with a mean age of 56.0 ± 24.3 years. They were all diagnosed as having carbuncle and/or furuncle and patient 2 progressed to necrotizing fasciitis. We determined three strains isolated from patients 1–3 as USA300 clone by molecular epidemiological analyses (Figure 2). Both PVL- and TSST-1-positive sequence type (ST) 22-SCC*mec* type IV strain was identified in patient 4. Patient 5 carried a PVL-positive ST59-SCC*mec* type V strain which called a Taiwan clone.[4] With the antimicrobial susceptibility test (EIKEN InoculaterΣ192), four isolates were resistant to levofloxacin and erythromycin but susceptible to clindamycin, minocycline, and fosfomycin as same as previous reports (Figure 2).[9] Gentamicin-resistant strains were found in three patients. Macrolide resistance genes (*ermC*, *msrA/B*, or *mphC*) and aminoglycoside resistance gene were detected in each resistant strains.

Clinical data of PVL-negative MRSA strains in four patients (patients 6–9) are shown in Table 1. The patients comprised two females and two males with a mean age of 54.0 ± 25.7 years. All patients had skin ulcers due to underlying intractable cutaneous and systemic disease and were diagnosed as having secondary MRSA infection. PVL-negative MRSA strains were detected in all skin ulcers but there was no evidence of carbuncle or furuncle. The proportion of furuncle/carbuncle in patients with PVL-positive MRSA (5/5 cases, 100%) was significantly higher than that in patients with PVL-negative MRSA (0/4, 0%; $p < .01$, Fisher's exact test). Severe cutaneous manifestations associated with the MRSA infections, such as necrotizing fasciitis, was only found in a patient with PVL-positive MRSA. Average durations of antimicrobial therapy in patients with PVL-positive MRSA were 14.67 ± 13.28 days by intravenous drip infusion (patients 1–3) and 14 days by oral antibiotics (patients 4 and 5). In contrast, that in patients with PVL-negative MRSA was 4.25 ± 2.99 days of oral antibiotics. No significant difference in gender, age, and laboratory data except the presence of bacteria was found between patients with PVL-positive and -negative MRSA.

4 | DISCUSSION

We experienced five patients with severe skin infections caused by PVL-positive MRSA in a short period (1.5 years) in Sendai, Japan. Notably, 3 of 5 (60%) PVL-positive MRSA strains were determined as USA300 clone. Suggesting that, PVL-positive MRSA, mainly USA300 clone, may spread in Tohoku, Japan. Severities of skin infection in patients with PVL-positive MRSA were clearly higher than those of patients with PVL-negative MRSA. Additionally, average durations of antimicrobial therapy for patients with PVL-positive MRSA (14.4 days) were 3.4-fold longer than those for patients with PVL-negative MRSA (4.3 days). USA300 clone is frequently associated with severe skin and soft tissue infections, particularly furunculosis, as well as severe life-threatening conditions such as necrotizing pneumonia.[10] Based on these findings, we should deal with PVL-positive MRSA as a causative agent for intractable skin infections in Japan likewise other countries.

We found both PVL- and TSST-1-positive ST22 clone, which recently emerged in Japan and named as ST22-PT clone.[11] We detected



Strain	SCC*mec*	TSST-1	PVL	ACME	ST (CC)	Resistance gene	Antimicrobial resistance
USA300	IV	-	+	I	8 (8)	ND	ND
Patient 1	IV	-	+	I	8 (8)	*msrA/B, mphC, aacA-aphD*	EM, LVFX, GM
Patient 2	IV	-	+	I	8 (8)	*msrA/B, mphC, aacA-aphD*	EM, LVFX, GM
Patient 3	IV	-	+	I	8 (8)	*ermC, msrA/B, mphC*	EM, LVFX
N315	II	+	-	-	5 (5)	ND	ND
Patient 4	IV	+	+	-	22 (22)	*ermC, aacA-aphD*	EM, LVFX, GM
Patient 5	V	-	+	-	59 (59)	None	None

FIGURE 2 Molecular epidemiological features of the MRSA strains isolated in this study. Molecular epidemiological analyses were performed by multilocus sequence typing, SCC*mec* typing, pulsed-field gel electrophoresis (PFGE), and PCR detection of virulence factors, such as PVL genes (*lukS/F-PV*), TSST-1 gene (*tst*), and ACME genes (*arcA* and *opp-3C*), and antimicrobial resistance factors, such as macrolide resistance genes (*ermA*, *ermC*, *msrA/B*, and *mphC*) and aminoglycoside resistance gene (*aacA-aphD*). PFGE types were characterized by more than 80% similarity. ACME, arginine catabolic mobile element; CC, clonal complex; EM, erythromycin; GM, gentamicin; LVFX, levofloxacin; ND, not determined; PVL, Panton–Valentine leukocidin; SCC*mec*, staphylococcal cassette chromosome *mec*; ST, sequence type; TSST-1, toxic shock syndrome toxin-1

TABLE 1 Clinical data of Panton–Valentine leukocidin-negative methicillin-resistant *Staphylococcus aureus* patients

	Age (years)	Gender	Underlying disease	Cutaneous lesion	Collection site	Oral antibiotics period
Patient 6	19	M	Palmoplantar pustulosis	Folliculitis	Hand	1 week
Patient 7	69	M	Follicutitis decalvans	Skin ulcer	Head	Topical only
Patient 8	77	F	Varicose ulcer	Skin ulcer	Leg	5 days
Patient 9	51	F	Polyarteritis nodosa	Skin ulcer	Leg	5 days

this ST22-PT clone in the cultures of a furuncle obtained from a Japanese patient with associated atopic dermatitis. The ST22-PT clone has been found in several healthcare facilities and increased prevalence in Japan (Kaneko et al. under manuscript submission). Thus, we should pay attention to trend of the prevalence of the ST22-PT clone in Japan. We also detected a PVL-positive ST59-SCC*mec* type V MRSA called Taiwan clone in Vietnamese patient. The Taiwan clone was frequently isolated from patients with severe disease.[12] Therefore, various PVL-positive MRSA clones associated with deep-seated skin infections may have been spreading in Sendai, Japan.

All PVL-positive MRSA clones isolated in this study exhibited highly susceptible to clindamycin, minocycline, and fosfomycin, which are available to use in community clinics. Therefore, we recommend rapid diagnosis and grasp effective antimicrobial agents for PVL-positive MRSA to prevent intractable skin infections.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, Tamihiro Kawakami, upon reasonable request.

ETHICS STATEMENT

Approval of the research protocol: Yes.
Informed Consent: Yes.
Registry and the Registration No. of the study/trial: N/A
Animal Studies: N/A

ORCID

Tamihiro Kawakami https://orcid.org/0000-0001-6741-939X

REFERENCES

1. DeLeo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated meticillin-resistant *Staphylococcus aureus*. Lancet. 2010;375:1557–68.
2. Kobayashi T, Nakaminami H, Ohtani H, Yamada K, Nasu Y, Takadama S, et al. An outbreak of severe infectious diseases caused by methicillin-resistant *Staphylococcus aureus* USA300 clone among hospitalized patients and nursing staff in a tertiary care university hospital. J Infect Chemother. 2020;26:76–81.
3. Iwabuchi C, Hino H, Shimada K, Kaneko H, Nakaminami H. Recurrent furunculosis by Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* USA300 clone in Tokyo: a report of two cases. J Dermatol. 2021;48:e522–3.
4. Takadama S, Nakaminami H, Sato A, Shoshi M, Fujii T, Noguchi N. Dissemination of Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* USA300 clone in multiple hospitals in Tokyo, Japan. Clin Microbiol Infect. 2018;24:1211.e1–7.
5. Takadama S, Nakaminami H, Aoki S, Akashi M, Wajima T, Ikeda M, et al. Prevalence of skin infections caused by Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* in Japan, particularly in Ishigaki, Okinawa. J Infect Chemother. 2017;23:800–3.
6. Nakaminami H, Ito A, Sakanashi D, Suematsu H, Yamagishi Y, Mikamo H, et al. Genetic diversity of pvl-positive community-onset methicillin-resistant *Staphylococcus aureus* isolated at a university hospital in Japan. J Infect Chemother. 2017;23:856–8.
7. Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, Etienne J, et al. Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: rapid identification system for mec, ccr, and major differences in junkyard regions. Antimicrob Agents Chemother. 2007;51:264–74.
8. Tenover FC, McDougal LK, Goering RV, Killgore G, Projan SJ, Patel JB, et al. Characterization of a strain of community-associated methicillin-resistant *Staphylococcus aureus* widely disseminated in the United States. J Clin Microbiol. 2006;44:108–18.
9. Nakaminami H, Ozawa K, Sasai N, Ikeda M, Nemoto O, Baba N, et al. Current status of Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* isolated from patients with skin and soft tissue infections in Japan. J Dermatol. 2020;47:1280–6.
10. Yokomori R, Tsurukiri J, Moriya M, Yamanaka H, Kobayashi T, Nakaminami H, et al. First report of fatal infection caused by community-acquired methicillin-resistant *Staphylococcus aureus* USA300 clone in a collegiate athlete. JMA J. 2020;3:78–82.
11. Yamaguchi T, Nakamura I, Sato T, Ono D, Sato A, Sonoda S, et al. Changes in the genotypic characteristics of community-acquired methicillin-resistant *Staphylococcus aureus* collected in 244 medical facilities in Japan between 2010 and 2018: a nationwide surveillance. Microbiol Spectr. 2022;10:e02272-21. https://doi.org/10.1128/spectrum.02272-21
12. Chen CJ, Unger C, Hoffmann W, Lindsay JA, Huang YC, Götz F. Characterization and comparison of 2 distinct epidemic community-associated methicillin-resistant *Staphylococcus aureus* clones of ST59 lineage. PLoS One. 2013;8:e63210. https://doi.org/10.1371/journal.pone.0063210

How to cite this article: Kawakami T, Yokoyama K, Ikeda T, Kaneko H, Watanabe Y, Nakaminami H. Severity and intractableness of skin infections caused by Panton–Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus*. J Cutan Immunol Allergy. 2023;6:94–97. https://doi.org/10.1002/cia2.12294